Product Manual

CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation), Trial Size

Catalog Number

CBA-130-T **24 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

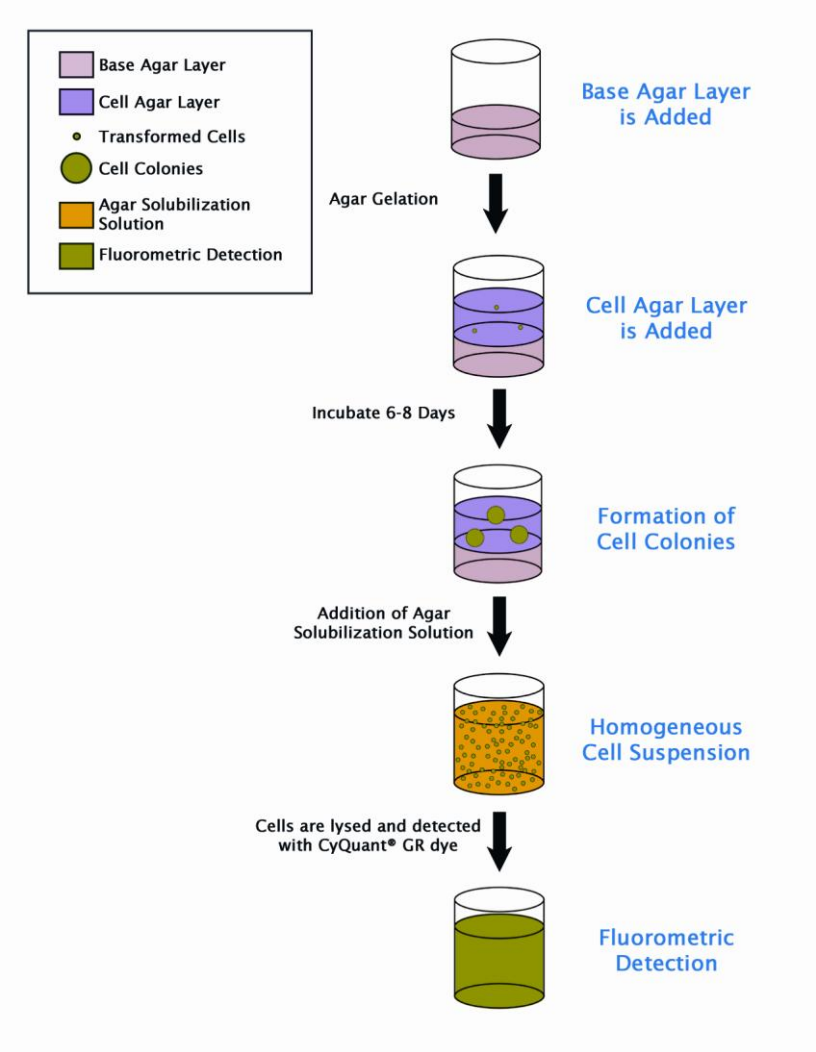
Neoplastic transformation occurs via a series of genetic and epigenetic alterations that yield a cell population that is capable of proliferating independently of both external and internal signals that normally restrain growth. For example, transformed cells show reduced requirements for extracellular growth promoting factors, are not restricted by cell-cell contact, and are often immortal. Anchorage-independent growth is one of the hallmarks of transformation, which is considered the most accurate and stringent in vitro assay for detecting malignant transformation of cells.

Traditionally, the soft agar colony formation assay is a common method to monitor anchorage-independent growth, which measures proliferation in a semisolid culture media after 3-4 weeks by manual counting of colonies. Standard soft agar assays are usually performed in 100-mm or 60 mm dishes, where cells are allowed to grow inside a semisolid culture media for 3-4 weeks before sizable colonies appear. This method is quite cumbersome, time-consuming, and difficult when testing a large number of samples. Additionally, the manual counting of colonies is highly subjective; with varying colony sizes, it's difficult to determine meaningful results.

Cell Biolabs CytoSelect™ 96-well Cell Transformation Assay does **not** involve subjective manual counting of colonies or require a 3-4 week incubation period. Instead cells are incubated only 6-8 days in a semisolid agar media before being solubilized, lysed and detected by the patented CyQuant® GR Dye in a fluorescence plate reader (see Assay Principle below). This format provides a quantitative, high-throughput method to accurately measure cell transformation. Additionally, the short incubation time (6-8 days) makes it possible to assay cells transiently transfected with oncogenes or siRNA.

The CytoSelect™ 96-well Cell Transformation Kit provides a robust system for screening oncogenes and cell transformation inhibitors. Each Trial Size kit provides sufficient quantities to perform 24 tests in a 96-well microtiter plate.

Assay Principle



Related Products

1. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
2. CBA-106-C: CytoSelect™ 96-Well Cell Migration and Invasion Assay (8µm, Fluorometric)
3. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
4. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
5. CBA-135: CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery, Colorimetric)
6. CBA-140: CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery, Fluorometric)
7. CBA-145: CytoSelect™ 384-Well Cell Transformation Assay
8. CBA-150: CytoSelect™ In Vitro Tumor Sensitivity Assay
9. CBA-155: CytoSelect™ Clonogenic Tumor Cell Isolation Kit
10. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

Kit Components

1. CytoSelect™ Agar Powder (Part No. 113001-T): One 0.24 g tube
2. 5X DMEM Solution (Part No. 113002): One 1.5 mL sterile tube
3. Agar Solubilization Solution (Part No. 113003-T): One 1.5 mL amber tube
4. 8X Lysis Buffer (Part No. 113004-T): One 1 mL tube
5. CyQuant GR Dye (Part No. 10103-T): One 10 µL tube

Materials Not Supplied

1. Cells and Culture Medium
2. 1X PBS
3. 37ºC Incubator, 5% CO_2 Atmosphere
4. Light Microscope
5. 96-well Fluorometer
6. Microwave or Heating Block
7. Water bath
8. (Optional) Positive Control cells such as NIH 3T3 (Ras G12V)

Storage

Store all components at 4ºC until their expiration dates.

Preparation of Reagents

- 1.2% Agar Solution: Place 0.24 g of Agar Powder in a sterile bottle, then add 20 mL of sterile cell culture grade water. Microwave or boil until agar is completely dissolved.
- 2X DMEM/20% FBS Medium: In a sterile tube, dilute the provided 5X DMEM in sterile cell culture grade water to 2X containing 20% FBS. For example, to prepare a 2.5 mL solution, add 1 mL of 5X DMEM, 0.5 mL of FBS and 1 mL of sterile cell culture grade water. Sterile filter the 2X media to 0.2 µm.

  Note: You may substitute your own medium in place of the DMEM we provide, but ensure that it is at a 2X concentration.
- CyQuant Working Solution: Immediately before use, prepare sufficient amount of the CyQuant Working Solution by diluting the CyQuant GR Dye 1:400 with 1X PBS. For example, add 10 µL to 4 mL of 1X PBS. Use the solution immediately; do not store the CyQuant Working Solution.

Assay Protocol (must be under sterile conditions)

I. Preparation of Base Agar Layer

1. Melt 1.2% Agar Solution in a microwave and cool to 37ºC in a water bath.
2. Warm 2X DMEM/20% FBS medium to 37ºC in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
3. Mix equal volumes of 1.2% Agar Solution and 2X DMEM/20% FBS medium in a sterile, pre-warmed tube by inverting several times. Immediately transfer 50 µL of the mixture to each well of a 96-well sterile flat-bottom microplate. Gently tap the plate a few times to allow the agar solution to evenly cover the wells.
   Notes:
   - *Work quickly with the agar solution to avoid gelation. Also, try to avoid adding air bubbles to the well.*
   - *To avoid fast and uneven evaporation that leads to aberrant results, we suggest not using the wells on the plate edge, or filling the edge wells with medium to reduce evaporation.*
4. Transfer the plate to 4ºC for 30 minutes to allow the base agar layer to solidify.
5. Prior to adding the Cell Agar Layer (Section II), allow the plate to warm up for 15 minutes at 37ºC.

II. Preparation of Cell Agar Layer (samples should be assayed in triplicate)

1. Melt 1.2% Agar Solution in a microwave and cool to 37ºC in a water bath.
2. Warm 2X DMEM/20% FBS medium to 37ºC in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
3. Harvest and resuspend cells in culture medium at 0.4 - 4 x 10^5 cells/mL, keep the cell suspension warm in a 37ºC water bath.
4. Mix equal volumes of 1.2% Agar Solution, 2X DMEM/20% FBS media, and cell suspension (1:1:1) in a sterile, pre-warmed tube by inverting several times. Immediately transfer 75 µL of the mixture to each well of the 96-well flat-bottom microplate already containing the solidified base agar layer (25 µL of cell suspension containing 1000-10000 cells/well will be seeded). *Note: Work quickly with the agar solution to avoid gelation, but gently pipette as not to disrupt the base layer integrity. Also, try to avoid adding air bubbles to the well. Always include negative control wells that contain no cells in the cell agar layer.*
5. Transfer the plate to 4ºC for 15 minutes to allow the cell agar layer to solidify.

III. Quantitation of Anchorage-Independent Growth

1. Add 100 µL of culture medium containing cell growth activator(s) or inhibitor(s) to each well.
2. Incubate the cells for 6-8 days at 37ºC and 5% CO_2. Examine the cell colony formation under a light microscope.

3. Remove culture medium by inverting the plate and blotting on paper towel. Gently tap several times.
4. Add 50 µL of Agar Solubilization Solution to each well of the 96-well plate. Incubate for 1 hr at 37ºC.
5. Pipette each well 5-10 times to ensure complete agar solubilization.
6. Add 25 µL of 8X Lysis Buffer to each well. Pipette each well 5-10 times to ensure a homogeneous mixture.
7. Incubate the plate at room temperature for 15 minutes.
8. Transfer 10 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
9. Add 90 µL of the CyQuant Working Solution to each well. Incubate 10 minutes at room temperature.
10. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

Cell Dose Curve (optional)

1. Harvest and resuspend cells in culture medium at 1 - 5 x 10^6 cells/mL.
2. Prepare a 2-fold serial dilution with culture medium, including a medium blank.
3. Transfer 125 µL of each cell dilution to a microfuge tube. Add 50 µL of Agar Solubilization Solution and 25 µL of 8X Lysis Buffer to each tube. Vortex each tube to ensure a homogeneous mixture. Incubate the tubes at room temperature for 15 minutes.
4. Transfer 10 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
5. Add 90 µL of the CyQuant Working Solution to each well. Incubate 10 minutes at room temperature.
6. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

Example of Results

The following figures demonstrate typical results with the CytoSelect™ 96-well Cell Transformation Assay Kit. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

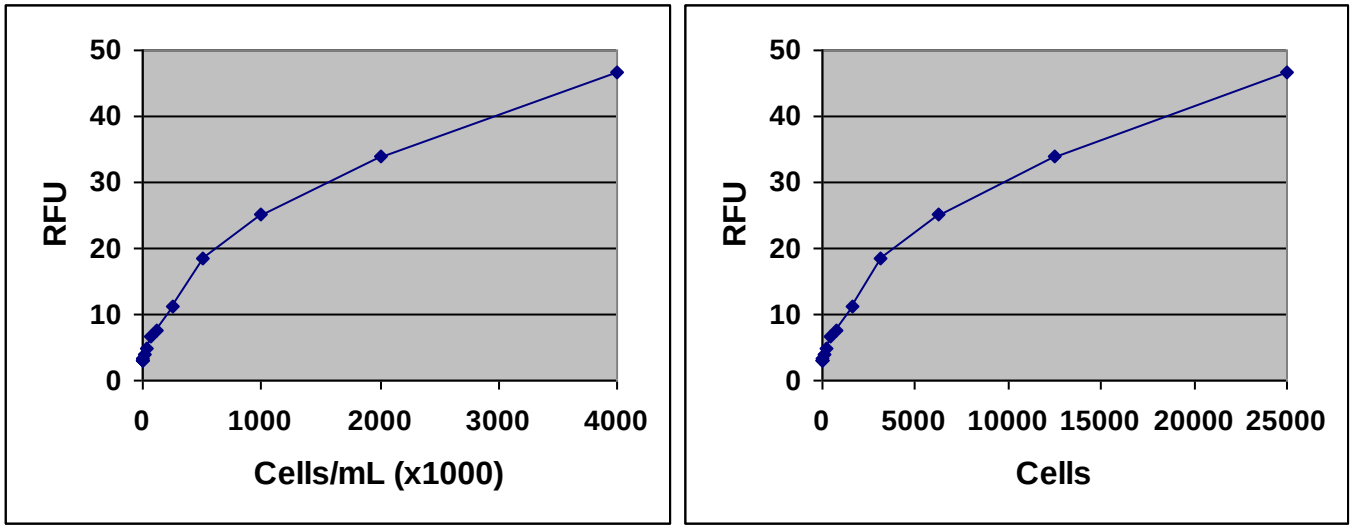


Figure 1. HeLa Cell Dose Curve. Cervical carcinoma HeLa cells were resuspended at 4 x 10^6 cells/mL and titrated 1:2 in culture medium, followed by addition of Agar Solubilization Solution, Lysis Buffer, and Cyquant® GR Dye detection (as described in the Cell Dose Section). Results were shown by cell concentration or by actual cell number in CyQuant Detection.

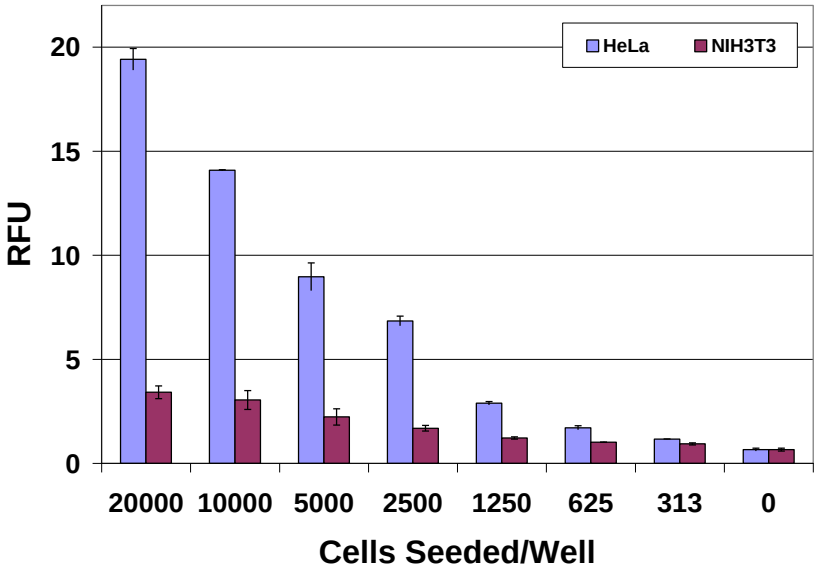


Figure 2. Anchorage-Independent Growth of HeLa Cells. HeLa cells were seeded at various concentrations and cultured for 6 days. HeLa cell transformation is determined according to the assay protocol.

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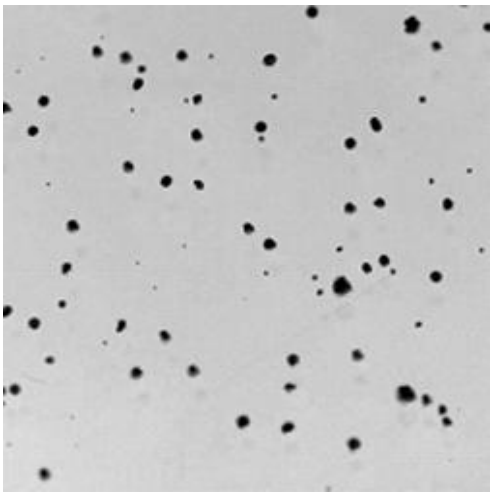

Figure 3. HeLa Colony Formation. HeLa cells were cultured for 14 days according to the assay protocol. Colonies were visualized by 0.1% p-iodonitro tetrazolium violet (INT) staining.

Calculation of Anchorage-Independent Growth

1. Compare RFU values with the Cell Dose Curve and extrapolate the cell concentration in soft agar.
2. Calculate the Total Transformed Cell Number/Well
   Total Transformed Cells/Well = cells/mL in soft agar x 0.125 mL/well

   For example: If you extrapolate your RFU value from your cell dose curve and determine you have 500,000 cells/mL in your soft agar sample.
   Total Transformed Cells/Well = 500,000 cells/mL x 0.125 mL/well = 62,500 cells/well

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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